

Bamboo and fungi

O. Schmidt*, D. S. Wei, T. K. H. Tang and W. Liese

Section of Wood Biology, Centre of Wood Sciences, University of Hamburg, Leuschnerstr. 91, 21031 Hamburg, Germany

Abstract: Bamboos are fast growing woody grasses and have an increasing importance for the sustainable production of materials with many applications. However, bamboo culms and products can be colonized and damaged by fungi and insects during storage, transport and final use. This review describes the isolation of moulds, their identification by molecular techniques as well as mould prevention using organic acids. Laboratory samples were discoloured by blue-stain fungi. Light microscopy showed the typical characteristics of blue-stain fungi in wood. Degradation by rot fungi was investigated on agar in preserving jars and on soil in the ‘Fungus cellar’. White-rot and soft-rot fungi decayed bamboo more than brown-rot species. Moisture from soil influenced decay. Chemical analyses of the cell wall components cellulose, hemicellulose and lignin reflected the obtained mass loss. Transmission electron microscopy showed a wood-like degradation pattern. UV microspectrophotometry of cell wall layers revealed attack on lignin by white-rot fungi.

Keywords: Moulding, Blue-staining, degradation, TEM, UV-microspectrophotometry

INTRODUCTION

Bamboos are impressive plants. They grow in warm climates all over the world. Within the Monocotyledoneae, Poales and Bambusoideae, 1317 woody bamboo species are described (Clark, 2012). Growth originates from a unique rhizome system (Liese, 1998). Monopodial species (‘running bamboos’) spread with their rhizome widely underground and send up new culms from the nodes. In sympodial species, the rhizome system forms at the clump. Unlike trees, there is no secondary growth of the culm. The culm emerges from the rhizome at its full diameter and grows 25 to 80 cm per day to its full height of maximum 15 to 30 meters in one growing season of 3-5 months. The culm is structured into mostly hollow internodal regions separated by nodes. Most bamboo species flower infrequently, at intervals of 30 – 80 years, exhibiting ‘mass flowering’ mostly with all plants of a particular species flowering worldwide over a period of several years. Thereafter they die. Classification is often assisted by the anatomical structure of the culm (Liese and Grosser, 2002). Cross

* To whom correspondence should be addressed; E mail: olaf.schmidt@uni-hamburg.de

sections show between the outer epidermis and the inner tissue the ground parenchyma with embedded vascular bundles consisting of vessels for upwards water and minerals transport and sieve cells for downwards carriage of assimilates.

Bamboos are the life basis for 1.5 million humans. Bamboo has highly economic and cultural significance, being used as a food source, as construction material and for many versatile products (Liese, 1985). The shoots and the rare fruits are edible. In rural areas, bamboo is used for cooking and heating. Like wood, bamboo is a natural composite material with a high strength-to-weight ratio useful for many purposes: as building material for constructions such as houses, bridges and furniture. Laminated and glued culm stripes are used for chairs and flooring (parquet).

However, bamboo has a low natural durability (Liese and Kumar, 2003). As a biological material like wood, bamboo in an endangered environment is susceptible to degradation by similar organisms which attack wood (Liese, 1959, 1985; Abdurachim, 1964; Mohanan, 1997; Razak *et al.*, 2006; Kleist *et al.*, 2002; Suprapti, 2010; Ma *et al.*, 2010; Kim *et al.*, 2011; Schmidt *et al.*, 2011; Wei *et al.*, 2012, 2013a, b). Insects deteriorate bamboo, moulds colonize it, staining fungi discolour and rot fungi degrade it. Bamboo species differ in durability. Samples from young culms and from the culm top, respectively, decay faster than older ones and those from the culm bottom (Schmidt *et al.*, 2011). The vulnerability of bamboo reduces the lifespan of its products and is a major hindrance to its applicability. Similar to wood, it is possible to safeguard bamboo against deterioration by protecting and preserving practices during storage and use. Bamboo for overseas transport and shorter use can be treated by short dipping or longer soaking in a solution of borax and boric acid. For longer protection, as needed for construction, the vertical soak and diffusion process, the sap-replacement pressure technique and the pressure impregnation can be used (Liese and Kumar, 2003).

The review summarizes results on the isolation of moulds from bamboo, their molecular identification as well as chemical protection measures against moulding, discolouration by blue-stain fungi, decay by rot fungi on agar and soil, chemical analyses of cellulose, hemicelluloses and lignin content as well as transmission electron microscopical and ultraviolet microspectrophotometric studies of degraded bamboo.

OCCURRENCE, IDENTIFICATION AND PREVENTION OF MOULDS

Bamboo is worldwide exported from the countries of origin. If not chemically preserved, the culms and their products can be colonized by moulds and insects during storage and sea transport in containers.

To investigate mould colonization, dry culms sections were kindly provided by colleagues worldwide. The bamboo species was mostly unknown. Moulds and blue-stain fungi (Deuteromycetes/Ascomycetes) were isolated from the culm surface by streaking spores and hyphae with an inoculation needle on 2% malt extract agar (MEA,

Oxoid), subcultured to pure cultures (Wei *et al.*, 2012) and identified by rDNA-ITS sequencing (Schmidt *et al.*, 2012). For the latter, DNA was extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR in the PTC-100 thermocycler (MJ Research, Watertown, MA, USA) with the Qiagen Taq Core Kit and the ITS1 and ITS4 primers of White *et al.* (1990) consisted of an initial denaturization of 4 min at 98°C, 35 cycles of 30 sec at 94°C for denaturization, 30 sec at 52°C for annealing, 1 min at 72°C for extension, and a final extension of 7 min at 72°C. Aliquots of PCR products were examined on 2% agarose gels (DNA agarose, Biozym, Hess. Oldendorf, Germany) with the Mupid-exU system (Advance, Tokyo, Japan). Gels were stained with GelStar Nucleic Acid Gel Stain (Cambrex, Rockland, ME, USA) and examined by UV light. PCR products suitable for sequencing were purified using the QIAquick PCR Purification Kit (Qiagen). Purified amplicons were sequenced in both directions by Eurofins Operon MWG (Ebersberg, Germany). Sequence identification was done by BLAST comparison with database sequences. Table 1 shows the identified fungi.

Most identified moulds and blue-stain fungi are common worldwide. There was no specific relation of fungal species to bamboo origin. Moulds colonize the surface of a broad spectrum of substrates by their spores that are present by chance in the air which surrounds the culms.

Due to the close relationships particularly among *Aspergillus* and *Penicillium* species, the identification result by ITS was controlled by sequencing the β -tubulin and calmodulin DNA. Identical results were obtained if these sequences had been deposited for a particular species. There were however also deviations, when compared with the ITS results, possibly due to errors in the databases.

For short-term protection of fresh culms during the mould-sensitive drying-down period, laboratory experiments with small samples were done in Hamburg (Tang *et al.*, 2009) and field tests with larger samples in South-Vietnam (Tang *et al.*, 2012). Samples were dipped for 10 min in 7 and 10% solutions, respectively, of various organic acids and salts. Small laboratory samples were inoculated with a conidia mixture of five deuteromycetes, *Aspergillus niger*, *A. flavus*, *A. oryzae*, *Epicoccum nigrum* and *Penicillium biourgeianum*, isolated in South-Vietnam from bamboo and DNA-identified in Hamburg. Fungi were incubated in plastic boxes for 8 weeks at 23°C. Larger culm sections for field tests became naturally infected by air spores for 1 day and were then covered for mould growth. Table 2 shows some results on mould prevention.

Acetic acid and propionic acid, respectively, completely prevented moulding, but their salts were ineffective (Tang *et al.*, 2009). The protection is obviously not only due to a particular chemical, but also influenced by the acid pH-value. The 2%/3% boron mixture of pH 8.7 was ineffective, which underlines observations in practice that boron-treated culms may not be free from moulds after container transport. After 8 weeks of storage in the natural climate (approx. 28°C, > 80 RH) in South-Vietnam,

Table 1. Identified fungi from bamboos

Bamboo origin	Deuteromycetes/Ascomycetes (number of strains)
China	<i>Alternaria alternata</i> (1)
	<i>Alternaria tenuissima</i> (1)
	<i>Arthrinium phaeospermum</i> (1)
	<i>Cladosporium cladosporioides</i> (2)
	<i>Dothiorella gregaria</i> (1)
	<i>Fusarium asiaticum</i> (1)
	<i>Fusarium culmorum</i> (1)
	<i>Fusarium zeae</i> (1)
	<i>Nigrospora oryzae</i> (4)
	<i>Penicillium commune</i> (1)
	<i>Penicillium chrysogenum</i> (1)
	<i>Penicillium tricolor</i> (1)
	<i>Penicillium variable</i> (1)
	<i>Phoma macrostoma</i> (1)
Germany	<i>Trichoderma koningiopsis</i> (2)
	<i>Trichoderma viride</i> (3)
Philippines	<i>Penicillium citrinum</i> (1)
	<i>Penicillium sumatraense</i> (1)
Thailand	<i>Aspergillus nomius</i> (1)
	<i>Aspergillus repens</i> (1)
	<i>Botryosphaeria subglobosa</i> (1)
	<i>Cladosporium cladosporioides</i> (2)
	<i>Epicoccum nigrum</i> (2)
	<i>Penicillium brevicompactum</i> (1)
	<i>Penicillium citrinum</i> (2)
	<i>Penicillium pinophilum</i> (1)
	<i>Trichoderma atroviride</i> (1)
Vietnam	<i>Trichoderma koningiopsis</i> (1)
	<i>Apiospora montagnei</i> (2)
	<i>Arthrinium phaeospermum</i> (1)
	<i>Arthrinium sacchari</i> (3)
	<i>Aspergillus flavus</i> (5)
	<i>Aspergillus niger</i> (2)
	<i>Botryosphaeria subglobosa</i> (5)
	<i>Epicoccum nigrum</i> (4)
	<i>Penicillium bialowiezense</i> (1)
	<i>Penicillium biourgeianum</i> (1)
	<i>Penicillium brevicompactum</i> (2)
	<i>Penicillium expansum</i> (1)
	<i>Penicillium islandicum</i> (1)
Total	<i>Pestalotiopsis microspora</i> (1)
	67

Table 2. Efficacy of chemicals against moulding of small bamboo samples (0 = no mould growth, 2 = 11-25 % surface coverage, 3 = 26-50 % coverage, 4 = > 51% coverage)

Chemical	%	<i>Bambusa tenostachys</i>		<i>Thyrsostachys siamensis</i>		pH- value
		Weeks of incubation		Weeks of incubation		
		1	8	1	8	
Control		3	4	3	4	
Acetic acid	10	0	0	0	0	2.8
Na-acetate	10	0	4	4	4	8.5
Citric acid	10	4	4	2	4	2.6
Formic acid	10	3	4	4	4	3.7
Propionic acid	7	0	0	0	0	2.9
Na-propionate	10	2	4	0	4	8.1
Boric acid +	2					
Na-borate	3	3	4	0	4	8.7

culm sections of *Bambusa procera*, *B. stenostachys*, *Dendrocalamus asper* and *Thyrsostachys siamensis* treated with propionic acid were also free from moulds (Tang *et al.*, 2012).

BLUE-STAIN TESTS

Blue-stain tests of small samples were performed with pure cultures from the laboratory strain collection and with some own isolates from bamboos on 2% MEA in Petri dishes for 1 to 5 months of incubation at room temperature. After the culture period, 20 μ m microtome sections were investigated under the light microscope (Wei *et al.*, 2012). Light microscopy showed the stained tissue colonized by the typical thick, brown hyphae and brown chlamydospores of blue-stain fungi (Liese, 1970, Schmidt, 2006). They occurred particularly in the vessels as this is the easiest way for these non-cell wall degrading fungi to colonize the tissue. Occasionally, a thin transpressorium penetrating a cell wall (Liese and Schmidt, 1964) was observed.

DEGRADATION TESTS

Samples were tested with the curcuma-test (Peylo, 2001) to ensure that no boron was present as commonly used against moulding during storage and transport. Different culture methods were applied to test degradation. The fungal isolates were derived from our laboratory strain collection or were own isolations from bamboo.

Decay experiments in preserving jars

Investigations were conducted with culm sections of *Bambusa maculata* and

Table 3. Bamboo degradation in preserving jars (average % mass loss of 3 replicates after 1 year incubation)

Fungus – isolate	Rot type	<i>Bambus amaculata</i>	<i>Gigantochloa atroviolacea</i>	<i>Phyllostachys pubescens</i>
<i>Pleurotus ostreatus</i> 11	white	28.2	10.6	21.0
<i>Trametes versicolor</i> 63	white	62.5	51.6	47.8
<i>Schizophyllum commune</i> 87	white	2.8	6.7	5.2
<i>S. commune</i> from bamboo	white	1.8	5.6	4.4
<i>Coniophora puteana</i> 167	brown	3.6	5.6	4.7
<i>Gloeophyllum trabeum</i> 183	brown	1.9	5.7	5.3
<i>Chaetomium globosum</i> 10	soft	31.8	9.4	38.0

Gigantochloa atroviolacea from Indonesia and *Phyllostachys pubescens* from Germany. Samples (3 x 1 cm; approx. 0.5 cm thick) were dried at 103°C, weighed and autoclaved. Household preserving jars with a volume of 500 ml were used as culture vessels and autoclaved for 20 min. MEA (110 ml per jar) was used for the Basidiomycetes and Abrams agar (Savory, 1954), supplemented with 0.1% yeast extract as vitamin source, for the soft-rot fungi. The jars were inoculated with fresh mycelial agar plugs (8 mm²) and incubated at 21°C and 70% RH for 1 year. The fungal mass loss was evaluated according to EN 113 (1996). Results are shown in Table 3.

Considerable decay occurred by the two white-rot fungi after one year of incubation, with a maximum 62.5% mass loss (ML) in *Bambusa maculata* by *Trametes versicolor*. Kolle flasks studies over 4 months according to EN 113 (1996) showed for *T. versicolor* ML from 2.3% in *Guadua angustifolia* to 15.3% in *Phyllostachys pubescens* (Wei *et al.*, 2013a). Zhang *et al.* (2007) studied 34 white-rot fungi and reported 13.6% ML in *P. pubescens*. Kim *et al.* (2011) measured up to 22% ML by six white-rot fungi in three *Phyllostachys* species. The soft-rot fungus *Chaetomium globosum* degraded *P. pubescens* samples to 38% (Table. 3). Various bamboo species showed ML by *C. globosum* of 7.2 to 47.2% after 6 month of incubation in Kolle flasks (Schmidt *et al.*, 2011). Kim *et al.* (2011) measured maximum 18% ML by soft-rot fungi. A Japanese isolate of *C. globosum* caused less decay (Suprati, 2010). The brown-rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* were less destructive similar like in EN 113 tests (Schmidt *et al.*, 2011; Wei *et al.*, 2013a). Low activity of *G. trabeum* however contrasts to 25% ML in *P. pubescens* (Lee *et al.*, 2006) and 35% decay of *P. edulis* (Ma *et al.*, 2010). The discrepancy may be caused by other isolates and culture methods. Although the white-rot fungus *Schizophyllum commune* is common on bamboos and wood (Liese, 1985; Mohanan, 1997), decay of bamboo (Table. 3) and wood (Schmidt and Liese, 1978) was low. There was no noteworthy decay difference between the laboratory isolate 87 of *S. commune* deriving from wood and isolate 98 from bamboo (Table. 3). Comparable results were observed in other studies (Suprati *et al.*, 2010; Kim *et al.*, 2011; Schmidt *et al.*, 2011). Abdurachim (1964) however

reported 15% ML. Leithoff and Peek (2001) found moderate decay by *S. commune* and *C. puteana* in Petri dishes and stronger decay by soft-rot according to the standard ENV 807 (2001).

The tested bamboo species differed in susceptibility to fungi (Table. 3; Wang and Hsieh, 1968; Hamid *et al.*, 2003; Suprati, 2010; Schmidt *et al.*, 2011). However, a standardized technique for bamboo sampling, test fungi and culture method is necessary. Susceptibility to fungi was influenced by the culm age as samples from young culms were more degraded than older ones (Hamid *et al.*, 2003; Schmidt *et al.*, 2011). Samples from the culm top with its higher starch content (Liese and Abd Latif, 2000; Okahishima *et al.*, 2007) were more decayed than from the bottom (Suprati, 2010; Schmidt *et al.*, 2011). The cutting season influences decay (Hamaguchi, 1953) because starch and protein content vary during the year (Magel *et al.*, 2006).

Decay tests in the Fungus cellar

To imitate natural conditions, the 'Fungus cellar test' was applied (Schmidt *et al.*, 2011; Wei *et al.*, 2012) used since long in Germany for basidiomycetes (Gersonde and Becker, 1958): Autoclaved samples (25 cm length) were placed on 30 l of unsterile garden soil in a metal container (120 cm length), either directly on soil or on sterile wood supports. After inoculation with pure cultures, the containers were covered with a glass plate to protect against drying. The soil was moistened weekly with sprayed tap water. Incubation at 23°C and 90% RH lasted 1 year. Results are given in Table 4.

Table 4. Mass loss (%) and final moisture (% u) in Fungus cellar test after one year incubation

Bamboo	Soil contact	<i>Coniophora puteana</i> 167		<i>Schizophyllum commune</i> 87	
		Mass loss	Moisture	Mass loss	Moisture
<i>Arundinaria amabilis</i>	-	38.6	41	10.8	31
	+	15.5	187	15.3	148
<i>Bambusa maculata</i>	-	20.4	39	5.1	28
	+	9.9	159	11.0	174
<i>Dendrocalamus asper</i>	-	29.3	42	4.3	28
	+	5.4	104	5.1	90
<i>Gigantochloa atrovioleacea</i>	-	18.7	34	4.7	25
	+	6.1	95	6.1	95
<i>Phyllostachys nigra</i>	-	32.6	49	9.1	32
	+	9.7	112	16.4	126
<i>Phyllostachys pubescens</i>	-	38.3	42	5.7	31
	+	6.3	61	6.3	63

The brown-rot fungus *C. puteana* decayed bamboo at a low moisture content, whereas the white-rot species *S. commune* needed a higher moisture content. Obviously, the moisture of the bamboo samples is important for degradation. Further factors may have influenced the fungi under these unsterile conditions such as nutrients or bacteria in the garden soil.

To imitate the conditions of the Fungus cellar test under sterile conditions, incubations were performed in preserving jars with vermiculite as reservoir for nutrients and different moisture (Wei *et al.*, 2013b). The results were unsatisfactory because the moisture range of the bamboo samples (35 to 100%) did not correspond to that in the Fungus cellar.

CHEMICAL ANALYSES OF DEGRADED BAMBOO

Incubated samples from the preserving jar experiments were chopped and gently pulverized in a vibration mill (HSM 100P, Herzog, Osnabrück, Germany). The bamboo flour was hydrolyzed in a 2-stage process with 72% and 4% sulphuric acid. Monomeric sugars were quantified by performance anion exchange chromatography (HPAEC)-borate technique (Willför *et al.*, 2009). Results are summarized in Table 5. The monomers 4-O-methyl-D-glucuronic acid, galactose, mannose and rhamnose are not included due to only low amounts of 0.1 – 0.2%.

Table 5. Chemical composition (%) of bamboos after 1 year of degradation in preserving jars

Bamboo	Fungus	Glucose	Cellulose	Cellulose	Arabinose	Xylose	Hemicellulose	Lignin
<i>Phyllostachys pubescens</i>	Control	41.5	0.7	42.2	1.4	24.4	26.8	26.2
	<i>Coniophora puteana</i> 167	41.1	0.2	41.3	1.2	23.8	26.0	25.6
	<i>Pleurotus ostreatus</i> 11	35.3	0.2	35.5	0.8	18.7	20.2	20.9
	<i>Trametes versicolor</i> 63	24.2	0.1	24.3	0.6	12.2	13.3	14.1
	<i>Schizophyllum commune</i> 87	39.7	0.2	39.9	1.0	23.1	25.0	25.8
	<i>Chaetomium globosum</i> 10	22.6	0.1	22.8	0.7	14.1	15.4	20.6
<i>Gigantochloa atroviolacea</i>	Control	47.8	0.7	48.5	1.2	16.1	18.6	29.5
	<i>Coniophora puteana</i> 167	47.8	0.2	48.0	0.8	15.7	17.6	27.7
	<i>Trametes versicolor</i> 63	25.0	0.1	25.1	0.4	7.1	8.0	14.4
	<i>Schizophyllum commune</i> 87	46.6	0.2	46.7	1.0	15.6	17.6	28.4

The composition of the two non-inoculated bamboo species was 42 - 49% cellulose, 19 - 27% hemicellulose and 26 - 30% lignin. As a consequence of the low mass loss, *C. puteana* and *S. commune* caused only a small decrease of cell wall components, whereas considerable decay of all three components occurred through the white-rot fungi *P. ostreatus* and particularly *T. versicolor* as simultaneous white-rot fungi. With regard to soft rot in bamboo (Liese, 1959; Sulaiman and Murphy, 1992), *Chaetomium globosum* showed consumption of cellulose, hemicellulose and even lignin. Lignin decrease may be due to demethoxylation of aromatic rings and cleavages in the side chains of the phenylpropane units (Schmidt, 2006).

TRANSMISSION ELECTRON MICROSCOPIC (TEM) AND ULTRAVIOLET MICROSPPECTROPHOTOMETRIC (UMSP) STUDIES

Sections for TEM and UMSP were taken from the first 0.5 mm of the fungi-infected sample surface. Specimens were fixed in glutaraldehyde/paraformaldehyde solution, dehydrated in a graded series of acetone and embedded in Spurr's epoxy resin to stabilise the degraded cell wall structure and facilitate the cutting procedure. Ultrathin sections of about 0.1 μm were prepared with an ultramicrotome for TEM. For UMSP, the embedded specimens were cut with an ultramicrotome using a diamond knife, whereby the thickness of cross sections was 1 μm . They were then transferred to quartz microscope slides, thermally fixed, mounted in non-UV-absorbing glycerol and covered with a quartz cover slip (Koch and Kleist, 2001; Schmitt *et al.*, 2006).

TEM studies

TEM provides general information and insight into morphological changes of the cell wall structure during decay. Lee *et al.* (2006) and Cho *et al.* (2008) investigated bamboo

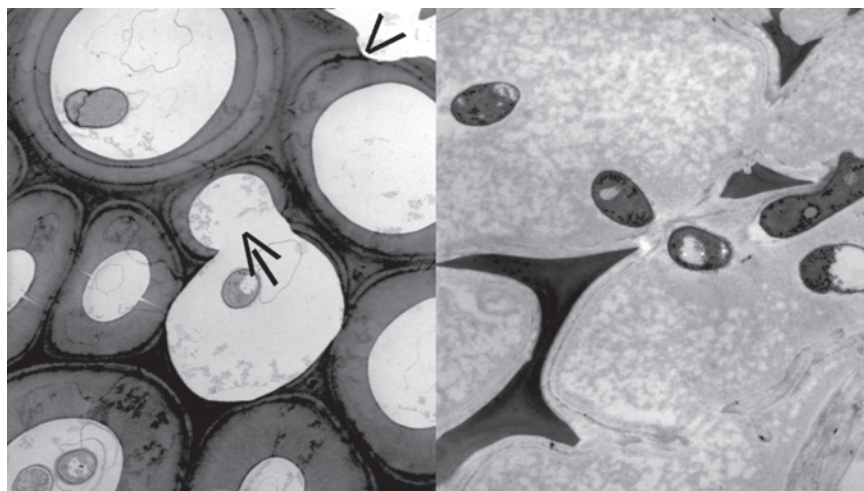


Figure 1. TEM pictures of white-rot in *Bambusa maculata* fibres. Left: initial decay by *Trametes versicolor* with erosion ($\uparrow$) and wall decay ($\downarrow$); right: advanced decay by *Pleurotus ostreatus* with completely decayed walls.

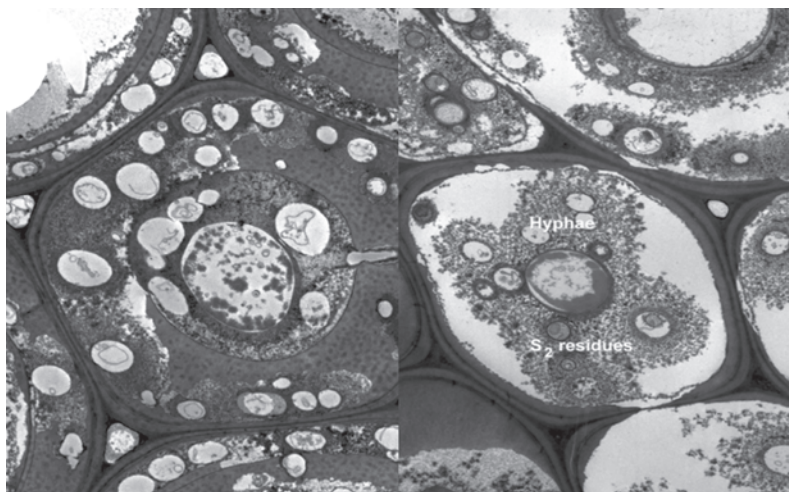


Figure 2. TEM pictures of soft-rot by *Chaetomium globosum* in *Bambusa maculata* fibres. Left: initial decay; right: advanced decay.

degradation by *Gloeophyllum trabeum* and Kim *et al.* (2008) by *Lentinula edodes*. White-rot degradation is shown in Fig. 1 and soft-rot decay in Fig. 2.

Trametes versicolor and *Pleurotus ostreatus* as simultaneous white-rot fungi degrade cellulose, hemicelluloses and lignin in parallel and at a similar rate (Schmidt, 2006). Decay started from the hyphae in the cell lumen by erosion of the S_3 wall (Fig. 1 left) progressively through the S_2 till the middle lamella primary walls (Liese, 1970). Finally, only the compound middle lamella (CML) region, particularly the cell corners remained (Fig. 1 right).

Typically, the hyphae of soft-rot fungi (Ascomycetes and Deuteromycetes) penetrate from the cell lumina into the secondary wall and produce chains of cavities in the S_2 layer (Liese, 1959) which occurred on cross sections at initial decay as small holes (Fig. 2 left). With progressing degradation, cavities coalesced to greater hollows and only the S_3 wall and CML region remained due to higher lignification (Fig. 2 right). The granular material on the right side in Figs. 1 and 2 may be cell wall remnants and fungal slime.

UMSP studies

UMSP is a suitable technique to measure changes in lignin content and composition within different cell wall layers (Fergus *et al.*, 1969; Scott *et al.*, 1969; Bauch *et al.*, 1976; Fackler *et al.*, 2013). The UMSP area scans and additional point analysis measurements reveal insights into the topochemistry in situ. Koch and Kleist (2001) studied the S_2 layer of the fibre wall of *Phyllostachys edulis* observing a typical UV spectrum for bamboo with a guaiacyl peak at 280 nm and a shoulder between 310–

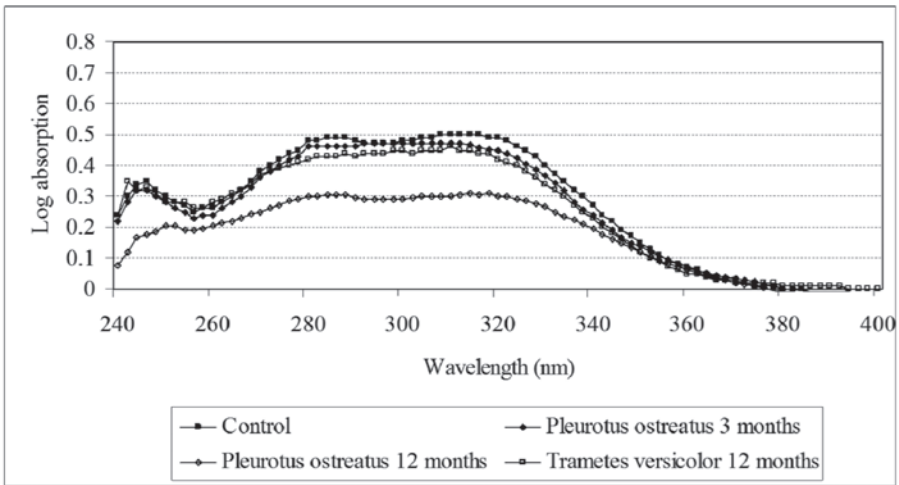


Figure 3. UMSP absorbance spectra of the vessel secondary wall of *Bambusa maculata* (average of 10 measurements).

315 nm due to the presence of *p*-coumaric acid esters. Cho *et al.* (2008) investigated *P. pubescens* fibres degraded by *Gloeophyllum trabeum*.

Figure 3 shows as an example of the various cell types and wall layers, the UV spectra of the vessel secondary wall of *Bambusa maculata* attacked by two white-rot fungi.

The curves in Fig. 3 have two maxima at about 280 nm and 315 nm wavelength. Both values decreased by the two white-rot fungi showing lignin degradation. Lignin drop by *P. ostreatus* increased with incubation time. Lignin decrease by these fungi was additionally visualized as UV microscopic scanning profiles (not shown) demonstrating the degradation patterns of the different cells and their wall layers.

CONCLUSION

Bamboos are impressive plants that grow in warm climates all over the world. As a life basis for 1.5 million humans, they have a highly economic and cultural significance, being used as a food source, as construction material and as a versatile product for many applications. Unlike trees, bamboo has only a low natural durability. In an endangered environment and without protecting measures bamboo is susceptible to easy degradation. Insects deteriorate bamboo, moulds colonize, staining fungi discolour and rot fungi decay it. Fungal impact was shown by isolation of moulds from bamboo, their molecular identification, chemical protection measures against moulding, discolouration by blue-stain fungi, decay by rot fungi, chemical determination of cell wall components and TEM and UMSP analyses.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr. Jürgen Puls, Dr. Uwe Schmitt and Dr. Gerald Koch for help with the chemical and microscopical investigations.

REFERENCES

- Abdurachim, M.R.A. 1964. Bamboo preservation in Indonesia. *Rimba Indonesia* 9: 66-76.
- Bauch, J., Seehann, G. and Fitzner, H. 1976. Microspectrophotometrical investigations on lignin of decayed wood. Suppl. 3 to *Material Organisms* 141-152.
- Cho, C.H., Lee, K.H., Kim, J.S. and Kim, J.S. 2008. Micromorphological characteristics of bamboo (*Phyllostachys pubescens*) fibers degraded by a brown rot fungus (*Gloeophyllum trabeum*). *J. Wood Sci.* 54: 261-265.
- Clark, L. 2012. An updated tribal and subtribal classification of the bamboos (Poaceae: Bambusoideae). Proc. IXth World Bamboo Congr., Antwerp, Belgium, 3-27.
- EN 113. 1996. Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin.
- ENV 807. 2001. Wood preservatives – determination of the effectiveness against soft-rotting micro- and other soil inhabiting micro-organisms. Beuth, Berlin.
- Fackler, K. and Thygesen, L.G. 2013. Microspectroscopy as applied to the study of wood molecular structure. *Wood Sci. Technol.* 47: 203-222.
- Fergus, B.J., Procter, A.R., Scott, J.A.N. and Goring, D.A.I. 1969. The distribution of lignin in sprucewood as determined by ultraviolet microscopy. *Wood Sci. Technol.* 3: 117-138.
- Gersonde, M. and Becker, G. 1958. Prüfung von Holzschutzmitteln für den Hochbau auf Wirksamkeit gegen Pilze an praxisgemäßen Holzproben ("Schwammkeller"-Versuch). *Holz Roh- Werkstoff* 16: 346-357.
- Hamaguchi, T. 1953. The relation between the season of felling bamboo and its decay. *J. Japan. For. Soc.* 35: 85-87.
- Hamid, N.H., Abd Latif, M. and Sulaiman, O. 2003. Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congress 2003, Québec, Canada, www.fao.org/docrep/article/wfc/XII/0039-b4.htm.
- Kim, J.J., Lee, S.S., Ra, J.B., Lee, H., Huh, N. and Kim, G.H. 2011. Fungi associated with bamboo and their decay capabilities. *Holzforschung* 65: 271-275.
- Kim, J.S., Lee, K.H., Cho, C.H., Koch, G. and Kim, Y.S. 2008. Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. *Holzforschung* 62: 481-487.
- Kleist, G., Morris, I. and Murphy, R. 2002. Invasion and colonization of bamboo culm material by stain and decay fungi. *Intern. Res. Group Wood Preserv.* 10453.
- Koch, G. and Kleist, G. 2001. Application of scanning UV microspectrophotometry to localise lignins and phenolic extractives in plant cell walls. *Holzforschung* 55: 563-567.
- Lee, K.H., Cho, C.H. and Kim, Y.S. 2006. Micromorphology of bamboo fibers degraded by brown-rot fungus *Gloeophyllum trabeum*. *Intern. Res. Group Wood Preserv.* 10576.
- Leithoff, H. and Peek, R. 2001. Heat treatment of bamboo. *Intern. Res. Group Wood Preserv.* 40216.
- Liese, W. 1959. Bamboo preservation and soft-rot. *Food Agricult. Organiz. Rep. Governm.*

- India, FAO Document 1106, 37 p.
- Liese, W. 1970. Ultrastructural aspects of woody tissue disintegration. *Ann. Rev. Phytopathol.* 8: 231-258
- Liese, W. 1985. Bamboos - biology, silvics, properties, utilization. Schriftenr. Gesellsch. Techn. Zusammenarbeit, Doc. 180, 132 p.
- Liese, W. 1998. The anatomy of Bamboo culms. Intern. Network Bamboo Rattan, Beijing, T. R. 18, 204 p.
- Liese, W. and Abd Latif, M. 2000. The starch content of two Malaysian bamboos in relation to age, culm height, site and harvesting month. XII IUFRO Word Congr., Poster, Vol. 3; 261.
- Liese, W. and Grosser, D. 2002. An expanded typology for the vascular bundles of bamboo culms.. Proc. Intern. Congr. Bamboo 2000, Chiangmai, Thailand, 121-134.
- Liese, W. and Kumar, S. 2003. Bamboo preservation compendium. Intern. Network Bamboo Rattan, INBR Doc. 22, 231 p.
- Liese, W. and Schmid, R. 1964. Über das Wachstum von Bläuepilzen durch verholzte Zellwände. *Phytopathol. Z.* 51: 385-393.
- Ma, X., Jiang, M. and Qin, D. 2010. The invasion channels of damage fungi in bamboo lumber. *Intern. Res. Group Wood Preserv.* 10712.
- Magel, E., Kruse, S., Lütje, G. and Liese, W. 2006. Soluble carbohydrates and acid invertases involved in rapid growth of developing culms in *Sasa palmata* (Bean) Camus. *Bamboo Sci. Cult.* 19: 23-29.
- Mohan, C. 1997. Diseases of bamboos in Asia. Intern. Developm. Res. Cent., New Delhi.
- Okahishima, Y., Yoshimura, T., Sugiyama, J., Horikawa, E.Y. and Imamura, Y. 2007. Longitudinal and radial distribution of free glucose and starch in moso bamboo (*Phyllostachys pubescens* Mazel). *J. Bamboo Rattan* 6: 21-31.
- Peylo, A. 2001. Schnellanalyse von Holzschutzmitteln. *Schützen & Erhalten*, September 2001: 25-27.
- Razak, W., Mahmud, S., Tamizi, M. and Awang, A.M.Y. 2006. Durability performance of *Gigantochloa scorteichinii* through laboratory fungal decay tests. *Res. J. Microbiol.* 1: 198-202.
- Savory, J.G. 1954. Damage of wood caused by micro-organisms. *J. Appl. Microbiol.* 17: 213-218.
- Schmidt, O. 2006. Wood and tree fungi. Biology, damage, protection and use. Springer, Berlin, 344 p.
- Schmidt, O. and Liese, W. 1978. Biological variations within *Schizophyllum commune*. *Material Organisms* 11: 215-230.
- Schmidt, O., Wei, D.S. and Liese, W., Wollenberg, E. 2011. Fungal degradation of bamboo samples. *Holzforschung* 65: 883-888.
- Schmidt, O., Gaiser, O. and Dujesiefken, D. 2012. Molecular identification of fungi in the wood of urban trees. *Eur. J. For. Res.* 131: 885-891.
- Schmitt, U., Singh, A., Frankenstein, C. and Möller, R. 2006. Cell wall modifications in woody stems induced by mechanical stress. *New Zealand J. For. Sci.* 36: 72-86.
- Scott, J.A.N., Procter, A.R., Fergus, B.J. and Goring, D.A.I. 1969. The application of ultraviolet microscopy to the distribution of lignin in wood description and validity of technique. *Wood Sci. Technol.* 3: 73-92.
- Sulaiman, O. and Murphy, R. 1992. The development of soft rot in bamboo fibres. *Intern. Res. Group Wood Preserv.* 1572.
- Suprapti, S. 2010. Decay resistance of five Indonesian bamboo species against fungi. *J. Trop.*

- For. Sci.* 22: 287-294.
- Tang, T.K.H., Schmidt, O. and Liese, W. 2009. Environment-friendly short-term protection of bamboo against molding. *J. Timber Developm. Assoc. India* 55: 8-17.
- Tang, T.K.H., Schmidt, O. and Liese, W. 2012. Protection of bamboo against mould using environment-friendly chemicals. *J. Trop. For. Sci.* 24: 285-290.
- Wang, S.-F. and Hsieh, R.-Ch. 1968. Durability records of treated and untreated bamboo. *Coop. Bull. Taiwan For. Res. Inst.* 15: 26 p.
- Wei, D., Schmidt, O. and Liese, W. 2012. Susceptibility of bamboo to fungi. Proc. 9th World Bamboo Congr. Antwerp, Belgium, pp. 235-245.
- Wei, D., Schmidt, O. and Liese, W. 2013a. Durability test of bamboo against fungi according to EN standards. *Eur. J. Wood Prod.* (submitted).
- Wei, D., Schmidt, O. and Liese, W. 2013b. Method to test fungal degradation of bamboo and wood using vermiculite as reservoir for moisture and nutrients. *Maderas* 15, (in press).
- Willför, S., Pranovich, A., Tamminen, T., Puls, J., Laine, C., Suurnäkki, A., Saake, B., Uotila, K., Simolin, H., Hemming, J. and Holmbom, B. 2009. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides – A comparison between different hydrolysis and subsequent analytical techniques. *Indust. Crops Products* 29: 571-580.
- White, T.J., Bruns T., Lee S. and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (Eds.), PCR protocols. Academic Press, San Diego, 315-322.
- Zhang, X., Yu, H., Huang, H. and Liu, Y. 2007. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. *Intern. Biodet. Biodegrad.* 60: 159-164.